TITLE: Lactic acid bacteria producing low molecular weight antimicrobial compounds.

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ABSTRACT

Reuterin (β-hydroxypropionaldehyde) is a neutral, low molecular mass compound produced primarily by some *Lactobacillus reuteri* strains during the anaerobic fermentation of glycerol. This compound exhibits broad spectrum effects against fungi, protozoa, Gram-negative and Gram-positive bacteria such as *Staphylococcus aureus*, one of the most common pathogenic bacteria associated with food poisoning. Thus, the objective of this study was to verify if the *L. reuteri* strain LR92 (Sacco DSM 26866) is a producer of reuterin and if this metabolite is able to inhibit the growth of *S. aureus* ATCC 29231 and N315 (multiresistant isolate). For the production of the metabolite the pre-activated culture in *L. reuteri* in MRS broth was centrifuged and resuspended in 300 ml of sterile glycerol aqueous solution (200 mM) and incubated for 3 hours at 37 °C in anaerobic jar (Anaerogen®). Cells were removed by centrifugation (8000 x g, 10 minutes) and the supernatant sterilized by membrane filtration 0.22 mm (Millipore) to thereby obtain the crude extract (EB). The detection and quantification of reuterin production in EB was obtained through a standard curve of the acrolein. The preparation of the samples for antimicrobial activity tests were performed in EB through liquid-liquid partitions in the ratio 1: 2 (v / v) with organic solvents dichloromethane (FD) and ethyl acetate (FA), the aqueous fractions of each partition, AD and AA respectively, were also used for the analyzes. To quantify the antagonistic activity of the FD and FA fractions (concentration of 700μL) against the S. aureus strains, agar diffusion tests were performed and wells tests were conducted for the aqueous fractions. In the crude extract it was possible to detect the production of reuterin that presented a concentration of 1.21 mmol L^{-1}. In the disc tests only FD demonstrated antimicrobial activity against *S. aureus* N315 presenting a halo of 2.00 mm. In the aqueous fractions tests, AD wells showed antimicrobial activity against the two strains tested with halos of 15.00 mm for ATCC 29231 and 2.00 mm for N315. Thus, through these results, *L. reuteri* LR92 demonstrated that may be a promising strain for the production of reuterin, acting as a natural antimicrobial agent against *S. aureus.*

KEYWORDS: anaerobiosis, antimicrobial, *Lactobacillus reuteri*, reuterin, *Staphylococcus aureus*

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